

INFLUENCE OF NEUROMUSCULAR FATIGUE ON CO-CONTRACTION BETWEEN VASTUS MEDIALIS AND VASTUS LATERALIS DURING ISOMETRIC CONTRACTIONS

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Original scientific paper

UDC: 612.766:577.35:537.3:796.012.4

Abstract:

The aim of this study was to evaluate the effect of neuromuscular fatigue on vastus medialis and vastus lateralis co-contraction. Fifteen healthy young subjects performed an isometric leg extension test in two different condition phases: i) maximum test for determining the maximum voluntary isometric contraction, and ii) fatigue test for exercises executed at 50% of the maximum voluntary contraction in three distinct periods with seven-day intervals between the tests. To quantify the co-contraction between the vastus medialis and vastus lateralis muscles, the signals overlapped and the intersection area was calculated. The data reliability was verified with the intraclass correlation coefficient (ICC) and standard error measurement (SEM). Values of median frequency and root mean square for both muscles showed a significant difference between the beginning and the end of the test, which indicates occurrence of the neuromuscular fatigue. The median frequency and root mean square ICC values ranged from moderate (ICC .66) to high (ICC .74) reliability with low SEM both for vastus medialis and vastus lateralis. The co-contraction values in the beginning of the test varied from 0.76 to 0.77, with a moderate reliability (ICC .63) and with low SEM. In the neuromuscular fatigue condition, ICC values of co-contraction varied from .75 to .76, with low reliability (.14) and with low SEM. Therefore, there were no significant differences between the co-contraction behavior at the beginning and the end of the fatigue tests. Based on these results, it is understood that the neuromuscular fatigue does not alter muscle co-contraction between vastus medialis and vastus lateralis in maximum voluntary contraction tests.

Key words: *electromyography, muscles, reproducibility of results*

Introduction

Muscle co-contraction is characterized by simultaneous contractions of two or more muscles around a joint (Fonseca, Silva, Ocarino, & Ursine, 2001; Silva, et al., 2014). From the biomechanical point of view, co-contraction is important for joint stabilization, minimizing the effects of potential internal and external disturbances, and regulating joint load (Choi, 2003). On the patellofemoral joint, quadriceps muscle co-contraction, especially of the vastus medialis (VM) and vastus lateralis (VL) (Heiden, Lloyd, & Ackland, 2009) provide a coordinated pattern for knee extension, thus stabilizing the patellofemoral joint (Rainoldi, Bullock-Saxton, Cavarretta, & Hogan, 2008).

Neuromuscular fatigue is known as a decline in the ability to generate muscular force (Montes, Alves, Gomes, Dezan, & Gomes, 2011). The decrease of muscle force occurs mostly due to neu-

romuscular factors that may affect the pattern of motor control during exercise (Missenard, Mottet, & Perrey, 2008). This change in the pattern of motor control leads to decreased co-contraction levels, altering both the precision of movements and the joint stability (Missenard, et al., 2008).

The processes involved in muscle fatigue seem to cause central nervous system (CNS) regulations that reduce muscular co-contraction. The adjustments in the pattern of muscle activation seem to be a CNS strategy to maintain muscle strength and endurance. Therefore, changes in activation strategies can provide valuable information regarding the mechanisms that alter neuromuscular strength of fatigued muscles (Semmler, Ebert, & Amara-sena, 2013).

The measurement of co-contractions obtained by the analysis of the electromyographic (EMG) signal behavior have been widely used to assess

the quality of motor coordination, motor learning stage and the joint stability level (Fonseca, et al., 2001; Winter, 2009). Despite the potential of EMG as a tool to investigate the neural control of muscle coordination in both isometric and dynamic situations, muscle force and activation relationship may be skewed in dynamic contractions due to fascicle length variations. In this case, similar forces could be achieved at different activation levels if the muscle operates at different lengths (Bouillard, Jubeau, Nordez, & Hug, 2014). Thus, the issue that will be approached in our manuscript is maximal voluntary isometric contraction (MVIC) tasks.

As described previously, many studies have demonstrated the effectiveness of surface electromyography for the assessment of both muscle co-contraction (Fonseca, et al., 2001; Heiden, et al., 2009; Semmler, et al., 2013; Girard, Bishop, & Racinais, 2013) and neuromuscular fatigue (Dideriksen, Farina, & Enoka, 2010; Cifrek, Medved, Tonkovic, & Ostojic, 2009). However, to our knowledge, no studies investigating the influence of muscle fatigue on co-contraction of the VM and VL muscles during isometric contraction were found. Therefore, we propose a transversal study to analyze the influence of neuromuscular fatigue in the VM and VL muscles co-contraction behavior during the isometric knee extension where co-contraction of VM and VL muscles is presented in association with two conditions, with and without neuromuscular fatigue. Our hypothesis is that co-contraction is diminished at neuromuscular fatigue condition.

Methods

Sample group

Fifteen male subjects, aged 21.93 ± 2.40 years, with body weights of 80.26 ± 8.88 kg and 177.4 ± 2.58 cm of body height, were included in the study. The sample was comprised of subjects without neuromuscular or any other joint disorder in the dominant leg 18 months before the study. Prior to measurement protocol, all subjects visited the laboratory and were informed about the procedures. After a short explanation, all participants signed a consent form. All procedures involved in the study were previously approved by the local research ethics committee (FCT-UNESP Presidente Prudente), and obtained the assent no. 17/2009.

Instrumentation

For the acquisition of the EMG signal, two pairs of surface electrodes in bipolar configuration (model Meditrace[®], 3M[®], with catchments Ag/AgCl and 10mm diameter) were used. The reference electrode was positioned on the styloid process of the ulna. The electrode cable contained a 20x signal gain circuit with a Common Mode Rejection over 80dB and impedance of 1K Ω . An electrical stim-

ulator (model Nemesys 942, Quark[®]) with a pen-type electrode was used to locate the motor point of the VM and VL muscles with a Transcutaneous Electrical Nerve Stimulation (TENS) current. After identifying each motor point, the skin was cleaned and shaved with 70% alcohol. The electrodes were placed parallel, with a 20 mm gap around the motor point in the direction of the muscle fibers of each muscle belly (De Luca, 1997). To ensure the positioning of the electrodes on three days of data measurement, a standard electrode positioning system that consisted of an acetate sheet layout was used. The technique involved the drawing of the upper border of the patella, the anterior superior iliac spine and the motor points found for each muscle in the acetate sheet. To recover the exact electrode placement on the consecutive measurements, the acetate layout sheet of each subject was aligned to the patella and the anterior superior iliac spine references which revealed the electrode placement previously defined.

The EMG signals were captured on a signal conditioning module (model EMG BIO 1000[®] brand LYNX[®] Electronic Technology Ltda, São Paulo - SP, Brazil). In this module, two channels were configured to acquire EMG signals, with a gain end of 1000 times and acquisition sampling frequency of 4000 Hz. All signals were stored in Bioinspector 1.8 software (LYNX[®]) data files and the muscle fatigue was assessed from the force signal applied by the subject to a load cell (Model MM-100 Kgf, Kratos[®] Sao Paulo, SP, Brazil), which was synchronized with the EMG signals in an additional channel configured on the conditioning module, enabling a low pass digital filter of 100 Hz.

Experimental procedures

A subject was seated on a knee extensor table (VITTALY[®] brand, model convergent[®]), with adjustments of the backrest and the lever length of resistance to the dominant leg. The angle of the knee joint was limited to 60° to allow the greater force generation of the quadriceps (Pincivero, 2003). During the MVIC the contralateral limb was fixed to the table with a stripe band to avoid body misalignments.

Three MVICs of the knee extensor muscles were performed in order to identify the exercise level necessary to induce the fatigue of the VM and VL muscles; the MIVCs were six seconds long with a rest period of 10 minutes. The first and the sixth second of these contractions were excluded for the calculation of the MVIC average. One week later, the first fatigue protocol was performed and consisted of one knee extension submaximal contraction of 50% of the MVICs average amplitude previously acquired.

During the fatigue test, the subject was told to hold the pre-defined load until exhaustion and the

knee angle was set at 60°. The exhaustion was considered as the moment in which the subject had ceased the muscle contraction or when a decline above 10% in target load, represented by a 50% of the MVIC, occurred. After the test, if the coefficient of variation of 50% MVIC was greater than 5%, the test was considered invalid and was repeated afterwards.

To assist and control the load intensity, the force was monitored by a load cell with a real time visual feedback on the computer screen. The fatigue tests were performed at three different moments always separated by intervals of seven days.

Data processing

EMG signals collected during the fatigue tests were processed using an algorithm developed in MATLAB® routines. The data of the first and the last two seconds of the EMG signal of the fatigue tests were discarded from the analysis. A 4th order Butterworth digital band pass filter with a cutoff frequency between 20 to 500 Hz was applied to the EMG raw data. The signal was windowed with epochs of 1000 milliseconds and with overlaps of 500 milliseconds to calculate the root mean square (RMS) and the median frequency (MF), using the discrete Fourier transform.

Two time points were selected for the EMG analysis during fatigue – the beginning and the end of the test. Both the initial MF (Mfreq_i) and the initial RMS (RMS_i) were calculated considering the average of the first five samples (time window of 1.25 milliseconds) of each variable extracted from the beginning of the test. The same procedure was applied to the end of the test, the end MF (Mfreq_F) and the end RMS (RMS_F) were calculated by the average of the last five samples (time window of 1.25 milliseconds) of each variable taken from the end of the test. RMS and MF were normalized by RMS_i and Mfreq_i, respectively.

The co-contraction between VM and VL muscles, indicated by the s EMG signal, was calculated using the cross-correlation analyses described by Winter (2009). In summary, the EMG signals were band pass filtered at 20-500 HZ, rectified, then an RMS was applied to the EMG data that were then normalized to the RMS from the MVIC trials.

Statistical analysis

Gaussian distribution of all variables was tested with the Shapiro-Wilk normality test. To analyze the fatigue test stability, the variables RMS_i, RMS_F, Mfreq_i and Mfreq_F (Factors) from VM and VL (Levels) were selected. The within- and between-subject variance was analyzed using an analysis of variance (ANOVA). A mixed between-within-subjects ANOVA and Bonferroni's *post-hoc* tests, with one

categorical independent within-subject variable (three assessments) and one categorical independent between-subject variable (two muscles), were used to assess the RMS_F and Mfreq_F values between the three fatigue assessments. In addition, one-way repeated measures ANOVA and Bonferroni's *post-hoc* tests were used for the comparison of the co-contraction (at the beginning and at the end of the test) between the three assessments of the fatigue test. The data reported from ANOVA are the F values (with degrees of freedom), p values and the eta squared (η^2), wherein this last value can range from 0 to 1 and represents the proportion of variance in the dependent variable that is explained by the independent variable. The guidelines (Cohen, 1988) for interpreting the η^2 are: .01=small effect, .06=moderate effect, .14=large effect (Field, 2013).

Paired *t*-test (two-tailed) was applied to compare: 1) the co-contraction between the beginning and the end of the fatigue test (test onset vs test completion) and 2) the MF and RMS values between the beginning and the end of the tests to confirm the occurrence of muscle fatigue in the present study, in which 1 was the value adopted to perform the tests, once it indicated the normalized value from the beginning of the fatigue test to both RMS_F and Mfreq_F parameters. The data reported from *t*-test are the F values (with degrees of freedom), p values and the eta squared (η^2).

The intraclass correlation coefficients (ICC) were calculated using the 2,k absolute concordance model for a relative measure of reliability (Weir, Therapy, & Moines, 2005). ICC values in the range of 0.00–0.25 indicate little, if any, reliability; values from 0.26 to 0.49 indicate poor reliability; values from 0.50 to 0.69 indicate moderate reliability; values from 0.70 to 0.89 indicate high reliability and values from 0.90 to 1.0 indicate very high reliability (Kellis & Katis, 2008; Mathur, Eng, & Macintyre, 2005). The standard error of measurement (SEM) was calculated by the square root of the variance error. This procedure allows the same unit of measurement as the tested variable that was used to express the reliability in absolute values (Weir, et al., 2005), which indicates the precision of the measurement (Bolgla, Malone, Umberger, & Uhl, 2010). The lower the SEM, the better the reliability of the measurement (Kellis & Katis, 2008). In this study, the ICC and SEM were calculated for the values of RMS_F, Mfreq_F and co-contraction between VM and VL muscles obtained on the three days of fatigue testing.

For the descriptive analysis, the averages, standard deviations and coefficients of variation (CV) were also presented, representing a percentage that shows how the variables were dispersed around the mean. Statistical analyses were performed using SPSS 18.0 software (SPSS Inc.) adopting $\alpha \leq 0.05$.

Results

The results presented in Table 1 and Figure 1 demonstrate the validity of the experimental design regarding the induction of neuromuscular fatigue. As is evidenced in Table 1, there was a significant increase in RMS and a significant decrease in MF, which indicates the presence of neuromuscular fatigue of VM and VL during the experimental protocol (see statistical analysis in Table 2). The MF and RMS variables showed a moderate to high reliability (0.66–0.73 ICC values) associated with low SEM for the VM and VL, (0.40 and 1.47, respectively).

A mixed between-within-subject analysis of variance was conducted to assess the behavior of the RMS_F and $Mfreq_F$ values across the three occasion assessments. The analysis performed has not found a significant interaction between the assessments and muscles (RMS_F : $F_{(2,56)}=0.52$, $p=.59$, $\eta^2=0.02$; $Mfreq_F$: $F_{(2,56)}=0.59$, $p=.55$, $\eta^2=0.02$). There was no significant main effect for assessments (RMS_F : $F_{(2,56)}=2.64$, $p=.08$, $\eta^2=.09$; $Mfreq_F$: $F_{(2,56)}=2.27$, $p=.11$, $\eta^2=0.07$) as well as no significant main effect comparing the two muscles (RMS_F : $F_{(1,28)}=2.08$, $p=.16$, $\eta^2=0.07$; $Mfreq_F$: $F_{(1,28)}=0.76$, $p=.39$, $\eta^2=0.03$),

Table 1. Mean (M), standard deviation (SD), coefficient of variation (CV), intraclass correlation coefficient (ICC) in last five samples of fatigue test of EMG normalized median frequency (MF) and root means square (RMS) for both muscles, vastus medialis (VM) and vastus lateralis (VL)

Variable	Assessment 1		Assessment 2		Assessment 3		ICC	SEM
	M±SD	CV(%)	M±SD	CV (%)	M±SD	CV(%)		
MF VM	0.89 ± 0.08	8.98	0.85 ± 0.11	12.94	0.91 ± 0.14	15.38	0.74	0.40
MF VL	0.86 ± 0.06	6.97	0.83 ± 0.11	13.25	0.85 ± 0.07	8.23	0.66	0.94
RMS VM	1.43 ± 0.32	22.37	1.52 ± 0.45	29.60	1.48 ± 0.36	24.32	0.71	1.47
RMS VL	1.33 ± 0.34	25.56	1.51 ± 0.38	25.16	1.32 ± 0.32	24.24	0.73	1.26

Table 2. Statistical analysis of the median frequency (MF) and root means square (RMS) data between the beginning and the end of the fatigue tests for both vastus medialis (VM) and vastus lateralis (VL) muscles

	Assessment 1		Assessment 2		Assessment 3	
	VM	VL	VM	VL	VM	VL
Mfreq _i vs Mfreq _f						
t-value	-6.235	-10.411	-4.884	-7.087	-2.487	-8.209
df	14	14	14	14	14	14
p-value (t-test)	<.001*	<.001*	<.001*	<.001*	.026*	<.001*
eta squared	.58	.79	.46	.64	.18	.71
RMS _i vs RMS _f						
t-value	5.356	3.773	4.512	5.641	5.422	3.839
df	14	14	14	14	14	14
p-value (t-test)	<.001*	.002*	<.001*	<.001*	<.001*	.002*
eta squared	.51	.34	.42	.53	.51	.34

*Significant difference between beginning and the end of the fatigue test, $p<.05$.

suggesting no difference between the VM and VL muscles.

During exercise, the VM and VL showed high levels of muscle co-contraction (0.77 and 0.76, respectively) (Table 3). There was no significant main effect for assessments in the initial ($F_{(2,28)}=0.454$, $p=.64$, $\eta^2=0.03$) and the final ($F_{(2,28)}=1.36$, $p=.27$, $\eta^2=0.09$) co-contraction values of the fatigue tests, suggesting no differences across the days measurements. The analysis comparing the co-contraction values between the beginning and the end of the tests showed no differenc-

Table 3. Descriptive values of mean (M), standard deviation (SD), coefficient of variation and the values of intraclass correlation coefficient (ICC) and standard error measurement (SEM) of the co-contraction. The test onset (two seconds after the beginning of the test) and test completion (two seconds before the end of the test)

Different Days	Test onset		Test completion	
	M±SD	CV (%)	M±SD	CV (%)
Assessment 1	0.77 ± 0.02	2.59	0.75 ± 0.02	2.67
Assessment 2	0.76 ± 0.03	3.94	0.75 ± 0.04	5.33
Assessment 3	0.77 ± 0.03	3.89	0.76 ± 0.02	2.63
ICC	0.63		0.14	
SEM	0.34		0.22	

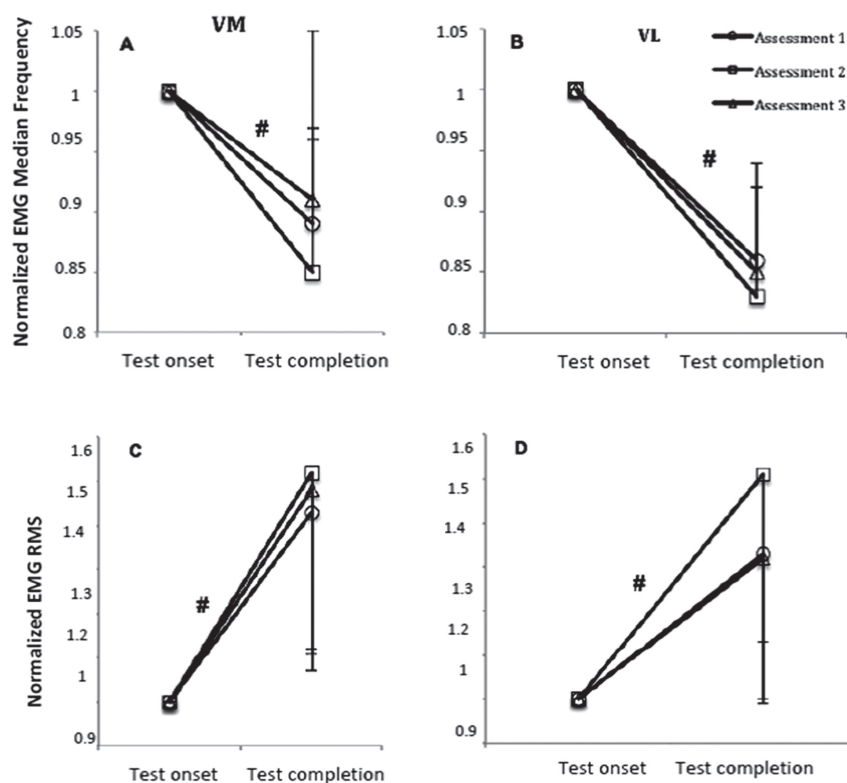


Figure 1. The average EMG normalized median frequency (MF) and RMS (A-B) and (C-D) in the first and last five samples of fatigue test to all three assessments. The data show the EMG MF and RMS for VM (A-C) and VL (B-D). # significant main effect (neuromuscular fatigue) for all three assessments at $p < .05$.

es for assessment 1 ($t_{(14)}=1.79$, $p=.09$), 2 ($t_{(14)}=1.55$, $p=.14$) and 3 ($t_{(14)}=0.94$, $p=.36$). The ICC values were .63 and .14 at the beginning and at the end of the tests, respectively, while comparing the three assessment days, associated with a relatively high SEM (0.34–0.22).

Discussion and conclusions

Muscle co-contraction is important for joint stabilization, minimizing the effect of potential internal and external disturbances, and for regulating joint load (Choi, 2003). Considering that the process of fatigue affects the pattern of motor control, it is believed that this condition could cause a decrease of muscle co-contraction (Missenard, et al., 2008). However, this hypothesis was not substantiated by our results.

In this study, co-contraction was assessed before and after fatigue induction. The results suggest that the VM and VL recruitment was similar throughout the exercise when the muscle co-contraction level was analyzed. Moreover, no significant difference was found between the beginning and the end of the tests, and among the assessment days. We consider that this condition is not related to the inefficiency of the fatigue protocol, but perhaps to a specific demand of the organism to make adjustments to preserve joint stability. The experimental protocol was efficient to induce fatigue since the increase in

RMS and the decrease in MF during a sustained contraction was evidenced (Table 1 and Figure 1), demonstrating condition in agreement with other studies (Yassierli & Nussbaum, 2008; Watanabe & Akima, 2010). Furthermore, the ICC values of the RMS and MF between assessments showed a moderate to high reliability associated with low SEM, indicating that the measurement of the RMS and MF was reliable and precise. Other authors have also demonstrated similar findings (Mathur, et al., 2005; Santos, et al., 2008; Callaghan, McCarthy, & Oldham, 2009) despite the different protocols used to induce neuromuscular fatigue. This evidence indicates that even in non-physiological working conditions, muscle co-contraction is maintained.

The simultaneous action of muscles around a joint promotes greater contact between joint surfaces, with a consequent increase in joint ability to withstand external loads (Markolf, Graff-Radford, & Amstutz, 1978). Some authors have proposed that joint stability would be achieved through the contribution of peripheral mechanoreceptors for continuous and dynamic adjustment of the co-contraction of muscles that act at the joint. These receptors communicate with the spinal gamma motoneurons, stimulating intrafusal fibers of the muscle spindle. The action of alpha motoneurons on the extrafusal fibers influences the muscle activation state, promoting the contraction of muscles around the joint. The resultant co-contraction from this mechanism

increases the articular stiffness and, consequently, improves joint stability (Johansson, Sjölander, & Sojka, 1991). In these circumstances, the low SEM indicates that the measurement is precise and, therefore, low ICC values of muscle co-contraction among the days of the testing procedure were found. Furthermore, neuromuscular fatigue could be explained by adaptations conditioned to this mechanism of action regarding the neuromuscular control. The results of this study show that these adaptations were necessary for the achievement of a high rate of co-contraction between the VM and VL above 75%, so that the subjects maintain the stability necessary to perform the tests, without any risk for joint stability.

Considering the results, it is suggested that further studies investigate the possible relationship between fatigue and neuromuscular behavior of joint stability in a dynamic condition of exercise. In addition, it is important that research proposals focus on experimental set-ups that aim at operationalizing

the analysis of the relationship of fatigue and neuromuscular co-contraction between VM and VL during the execution of known and unknown activities. We believe that these approaches have great potential for contributions to the field of physical activity, i.e. to health and rehabilitation, especially with regard to the conditions of exercises that promote the physiological process of patellofemoral joint stabilization.

The interpretation of the results showed that the VM and VL muscles were exposed to neuromuscular fatigue. However, fatigue did not change the levels of VM and VL muscles co-contractions during submaximal isometric contractions. The high values of co-contraction were associated with moderate to low reliability and low SEM. These findings suggest that the neuromotor system adaptations occurred before the changes caused by induced neuromuscular fatigue for an adequate maintenance of joint stability during the isometric exercise.

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Submitted: March 10, 2014

Accepted: September 30, 2014

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Acknowledgement

This research was supported by São Paulo Research Foundation (FAPESP), nº 2008/11639-4.